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SKELETAL MUSCLE METABOLISM IN HYPOKINETIC RATS

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Amino acid metabolism in hypokinesia. The previous report (3-1-82 to 8-31-83) presented data showing marked effects of hypokinesia on glutamine synthesis. In the soleus muscle, which responds to hypokinesia with significant atrophy, the synthesis of glutamine and glutamate were 63% less in the suspended non-weight bearing (SNWB) rats than in the suspended weight bearing (SWB) ones. This response was not altered by adrenalectomy. In the extensor digitorum longus muscle, which is not nearly as sensitive to reduced weight bearing, no effect on synthesis of glutamine and glutamate was seen. Synthesis of alanine was not affected in either muscle of rats with intact adrenals.

In muscle, a major source of nitrogen for the synthesis of these amino acids are the branched-chain amino acids, which transaminate with α-ketoglutarate to produce glutamate (Fig. 1). Indeed a major function of alanine and glutamine in muscle is to provide a vehicle for transporting nitrogenous waste, derived from amino acid degradation, out of the tissue. One posssible reason for the decreased synthesis of glutamine and glutamate might be decreased metabolism of the branched chain amino acids, which were present in the medium. To test this possibility, we measured the production of $^{14}CO_2$ and α-^{14}C-ketoisocaproic acid from [1-^{14}C]leucine in muscles of normal and hypokinetic rats. The sum of these products reflects flux through leucine aminotransferase and production of $^{14}CO_2$ is a measure of flux through α-ketoisocaproate dehydrogenase. In the soleus muscle, non-weight bearing for 6 days increased the rate of flux through leucine aminotransferase (Table 1). This effect could be accounted for by increased flux through α-ketoisocaproate dehydrogenase, the rate-limiting step in the leucine degradative pathway. No differences were seen for the extensor digitorum longus muscles over the same period. This result is contrary to the previous data for decreased

synthesis of glutamine. In fact, the data suggest that the soleus muscle could have a severe problem in removing nitrogen waste derived from the increased degradation of the branched-chain amino acids. We are currently pursuing this problem further by looking at the degradation of other amino acids and the reasons for the decreased synthesis of glutamine (e.g., decreased enzyme content).

As part of our study of leucine metabolism, we also considered to what extent leucine was oxidized completely. This can be done by comparing the production of $^{14}CO_2$ from [1-^{14}C] and [U-^{14}C] leucine. The former will yield the rate of irreversible leucine decarboxylation at the first carbon. Comparison of the two rates can then provide information as to the further oxidation of the intermediates in the pathway. The [^{14}C]acetyl CoA produced from leucine is diluted in the tissue by metabolism of unlabeled glucose and fatty acids to the same endproduct. Hence the $^{14}CO_2$ production from [U-^{14}C] leucine will always be underestimated significantly. If a ratio of only about 0.17 is obtained then none of the leucine molecule is completely oxidized. Since both soleus and extensor digitorum longus muscles showed ratios significantly above this value, leucine must undergo significant oxidation in these muscles. Hypokinesia did not affect the extent of leucine oxidation (Table 1). Therefore increased leucine catabolism in soleus muscles of hypokinetic animals would increase the supply of acetyl CoA oxidized in the citric acid cycle.

In line with our interests in amino acid metabolism, we have measured the urinary excretion of urea and free ammonia in normal non-suspended weight bearing (NSWB), control suspended weight bearing (SWB) and hypokinetic suspended non-weight bearing (SNWB) rats. During the first 4 days of suspension the gain in body weight was similar for all animals (Figure 2). On days

5 and 6, both the SWB and SNWB groups showed a slower gain in body weight, apparently due to the stress of suspension. Thereafter, the SWB group gained less than the normal (NSWB) rats and the SNWB group gained less than either of the other groups. Despite the slower rate of weight gain, the suspended rats excreted urea and ammonia at the same rate as normal rats (Figure 3). In accord with this result, we found that the rate of food consumption/100 g body weight did not differ between the various groups.

Effects of passive stretch of muscles in hypokinesia. The most critical problem to be resolved is how to prevent muscle atrophy and loss of muscle function under decreased weight bearing conditions. It is well known that passive stretch of muscles can induce hypertrophy. Therefore, we tested whether it might also reverse the effects of atrophy in hypokinesia. For this study, we used SWB and SNWB rats as before. To test the effect of passive stretch in the hypokinetic rats, we casted one leg in a dorsal flexed position, which stretches the soleus, gastrocnemius and plantaris muscles, while shortening the extensor digitorum longus and tibialis anterior muscles. Measurements of muscle weight and protein metabolism were taken for muscles from the SWB rats and from the free moving and dorsal-flexed (casted) legs of the hypokinetic animals.

Comparison of the free-moving leg of hypokinetic rats with weight bearing leg muscles shows the effect of reduced weight bearing (Table 2). The soleus muscle was 31% smaller, the gastrocnemius 15% smaller and the plantaris 7% smaller in the legs of suspended non-weight bearing animals. The extensor digitorum longus and tibialis anterior were unaffected by 6 days of hypokinesia. For the soleus muscle, the muscle from the dorsal-flexed leg was 2-fold larger than from the free moving leg and was even larger by 44% than the weight bearing muscle. Therefore dorsal flexion (passive stretch) not only prevented atrophy of the soleus but actually

induced hypertrophy relative to the weight bearing muscle. For the plantaris and gastrocnemius, dorsal flexion prevented the atrophy in hypokinesia but did not induce hypertrophy of these muscles. In the shortened muscles, the tibialis anterior showed a slight loss of mass (3%). The loss in the extensor digitorum longus was not significant. Therefore, on a weight basis several muscles increased in size and others were not adversely affected.

For the soleus and extensor digitorum longus muscles, we compared rates of protein synthesis and degradation for the free-moving and dorsal-flexed legs. The hypertrophy of the soleus muscle in passive stretch was accompanied by a rise in protein synthesis (44%) and a fall in protein degradation (26%) as measured in vitro. In accord with no change in the muscle weight, the extensor digitorum longus showed no differences in the rates of protein synthesis and degradation in the dorsal-flexed muscle (Table 3).

Although dorsal-flexion prevented muscle atrophy, in several leg muscles of the hypokinetic rats, we do not know as yet whether muscle function is maintained at normal levels. We plan to test this question by comparing biochemical parameters in dorsal flexed and free-moving limbs. Preliminary results suggest, for instance, that dorsal flexion may prevent the marked decrease in glutamine synthesis seen in soleus muscles of hypokinetic animals.

BIBLIOGRAPHIC REFERENCES

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Jaspers, S.R., Fagan, J.M. and Tischler, M.E. (1983) Effect of limb immobilization on protein turnover and redox state in muscles of hypokinetic rats. (Abstract) Fed. Proc., in press.
(To be presented at the ASBC meeting in June, 1983)

TABLE 1

EFFECTS OF HYPOKINESIA ON LEUCINE METABOLISM IN LEG MUSCLES OF RATS

Condition	Enzyme Flux (nmol/mg tissue/2h)		Production of $^{14}CO_2$ from ^{14}C-Leucine
	Leucine Transaminase	Ketoisocaproate Dehydrogenase	Ratio for $[U\text{-}^{14}C]/[1\text{-}^{14}C]$
		SOLEUS	
Suspended weight bearing	0.230 ± 0.015	0.161 ± 0.011	0.387 ± 0.040
Suspended non-weight bearing	0.298 ± 0.020	0.231 ± 0.013	0.388 ± 0.029
Difference (%)	30 ± 11	43 ± 11	0
	$p<0.05$	$p<0.01$	NS
		EXTENSOR DIGITORUM LONGUS	
Suspended weight bearing	0.354 ± 0.024	0.22[illegible] ± 0.021	0.466 ± 0.050
Suspended non-weight bearing	0.370 ± 0.027	0.268 ± 0.027	0.452 ± 0.029
Difference (%)	5	17	3
	NS	NS	NS

NS = not significant difference

TABLE 2

EFFECTS OF WEIGHT BEARING AND DORSAL FLEXION ON WEIGHTS OF LEG MUSCLES

Muscle	Weight Bearing	Non-Weight Bearing	
		Free Moving	Dorsal Flexed
	(g/100 g body wt)		
Soleus	39 ± 1	27 ± 1[a]	56 ± 1[a,b]
EDL	49 ± 1	49 ± 1	46 ± 1
Plantaris	86 ± 2	80 ± 1[a]	86 ± 2[b]
Gastrocnemius	459 ± 6	392 ± 6[a]	470 ± 7[b]
Tibialis Anterior	198 ± 4	196 ± 3	190 ± 3[a,b]

[a]Significant difference from weight-bearing.

[b]Significant difference from free moving.

TABLE 3

EFFECTS OF DORSAL FLEXION ON PROTEIN METABOLISM IN LEG MUSCLES OF HYPOKINETIC RATS

	Protein Synthesis		Protein Degradation	
	Soleus	EDL	Soleus	EDL
	(nmol/mg muscle/2h)			
Free Moving	0.061±0.003	0.051±0.002	0.383±0.018	0.220±0.013
Dorsal Flexed	0.088±0.004[a]	0.046±0.002	0.282±0.006[a]	0.230±0.018

[a]Significant difference from free moving leg.

FIGURE 1

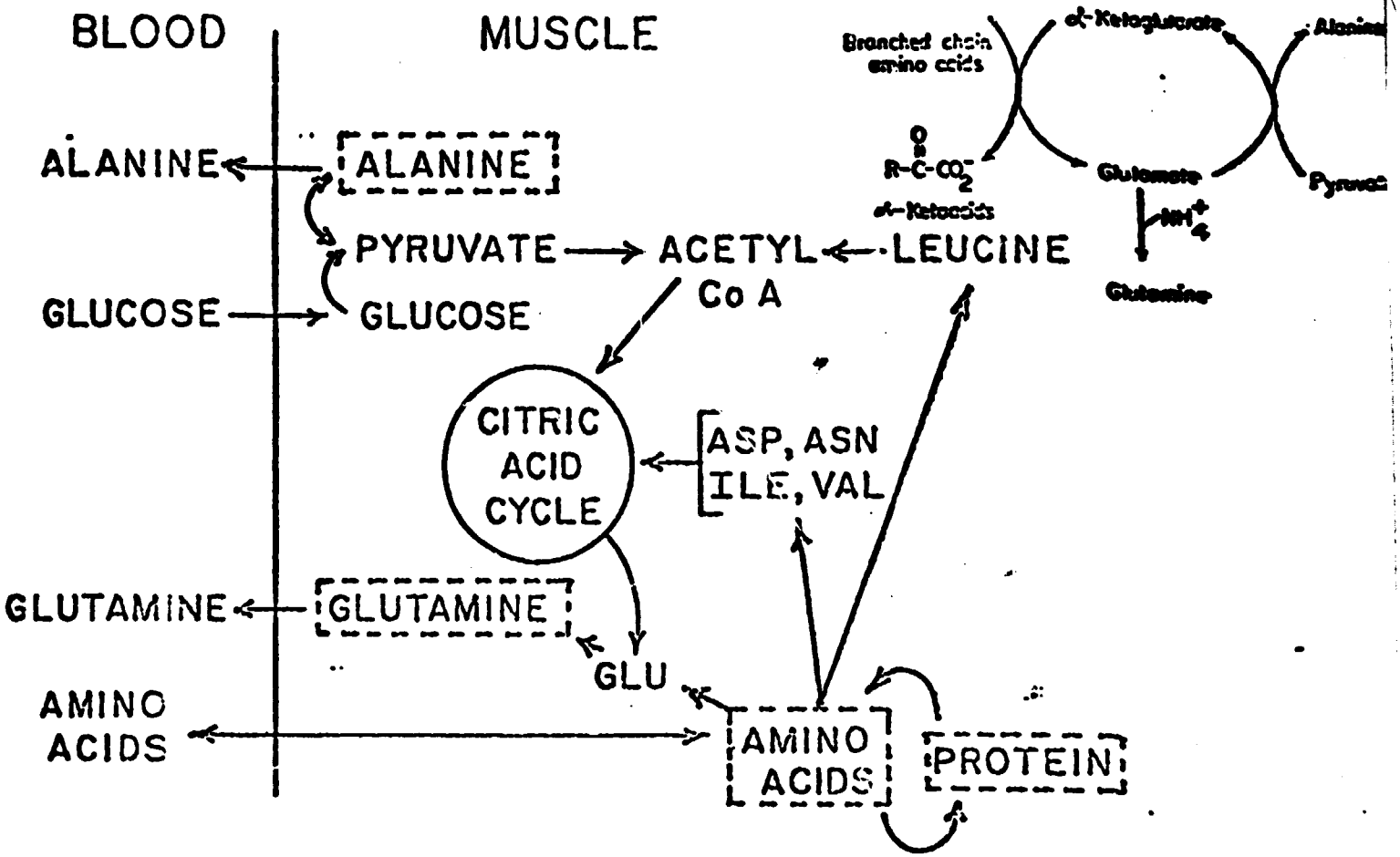

FIGURE 2

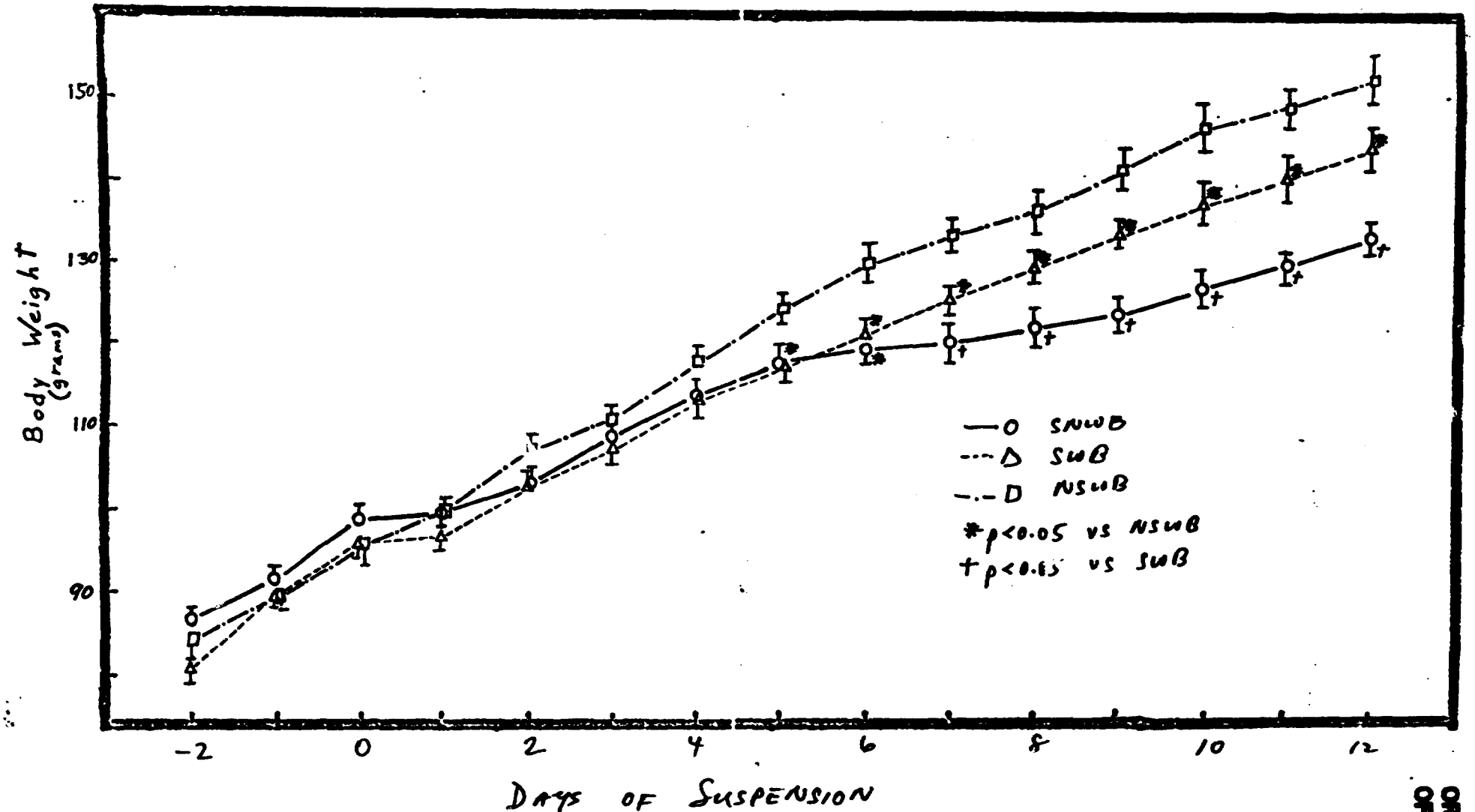

FIGURE 3